Product Technical Note

Custom Oligo Synthesis, siRNA, antisense oligos, RNA oligos, chimeric oligos, Fluorescent probes, Modified oligos

Oligo Stability, Reconstitution and Storage

DNA, siRNA, Fluorescent, RNA & modified Oligo

For Research Use Only. Not for use in diagnostic procedures for clinical purposes

Oligonucleotide Stability, Reconstitution and Storage

All Gene Link custom oligo products including molecular probes, RNA, fluorescently modified, ASO, siRNA and complex chimeric oligos include a datasheet that contains the exact nmols, µg, A_{260} units(OD Units) and other physical data. This data is important for reconstituting the product. All oligos are shipped dried/lyophilized unless requested to be shipped reconstituted.

All oligos tubes before opening should be briefly centrifuged to bring the dried oligos to the bottom of the tube. The dried oligos during shipment get dislodged and may be on the inside of the cap of the tubes and inadvertently may get lost while opening the tube.

Unmodified Oligos Stability, Reconstitution & Storage

Reconstitution
Gene Link unmodified oligos are supplied dried/lyophilized. These are stable at room temperature for an extended period, up to several years. The oligonucleotide should preferably be stored frozen upon receipt. TE buffer (10mM Tris, 1mM EDTA, pH 8.0) is recommended for dissolving the oligonucleotides; EDTA inhibits the activity of the nucleases.

Further dilution can be made in low TE buffer (10mM Tris, 0.1mM EDTA, pH 8.0). After reconstitution, store the stock solution at -80°C or -20°C.

Oligos can also be dissolved in nuclease free water if required. The pH of water should be between 7-8. Acidic water leads to depurination of oligos.

On occasion certain oligos with high GC content or secondary structure may not dissolve rapidly. Heating at 55°C usually is sufficient to dissolve these oligos. Addition of DMSO gradually from a concentration of 1% to maximum concentration of 10% is recommended for oligos that do not dissolve even at 55°C.

Storage
Further dilution can be made in low TE buffer (10mM Tris, 0.1mM EDTA, pH 8.0). After reconstitution, store the stock solution at -80°C or -20°C.

Stability
Unmodified oligos are generally stable for 2 years or longer if reconstituted and stored appropriately as recommended by Gene Link. The stability can be increased several fold by instituting proper handling conditions, avoiding exposure to light and multiple freeze thaws.

Preparation of Stock Solution of 100 pmols/µl [100µM]
Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of TE to be added.
Example: 45.10nmols x 10 = 451 µL
Dissolve the oligo in 451 µL to get 100pmols/µl stock solution.
Use as required.
Dilute 10 fold to prepare a 10pmols/µl [10µM]. Use as required.

Fluorescently modified Oligos Stability, Reconstitution & Storage

Reconstitution

The dried oligo during transportation may have dislodged from the bottom of the tube and may reside in the cap and/or distributed on the sides of the tube.

Prior to opening the tube, briefly centrifuge the tubes to ensure that the dried oligonucleotide is at the bottom of the tube. Resuspend single stranded siRNA oligonucleotides at a convenient concentration, e.g. 100 µM, in RNase free sterile water and duplex siRNA at a concentration of 100 µM. These are general guidelines, and you may elect to reconstitute at a different concentration or buffer condition.

All fluorescently modified oligos are shipped in amber tubes to prevent exposure to light and minimize photobleaching. Gene Link oligos are supplied dried/lyophilized. These should preferably be stored frozen upon receipt. Refer to the table below for specific TE buffer pH for certain fluorescent dyes. TE (10mM Tris, 1mM EDTA, pH7 to 8.0) is recommended for dissolving the oligonucleotides; EDTA inhibits the activity of the nucleases.

Preferred TE Buffer Reconstitution & Storage pH for Fluorescent Probes	
6-FAM, HEX, TET, ROX, and TAMRA	TE Buffer pH 7.5 or 8.0
Cy3, Cy3.5, Cy5, and Cy5.5	TE Buffer pH 7.0 or 7.5
Cy dyes rapidly degrade in acidic pH	

Further dilution can be made in low TE buffer (10mM Tris, 0.1mM EDTA, pH 7 to pH8.0). After reconstitution, store the stock solution at -80°C or -20°C. Fluorescently labeled oligos should be stored in light-free conditions.

Oligos can also be dissolved in nuclease free water if required. The pH of water should be between 7-8. Acidic water leads to depurination of oligos.

On occasion certain oligos with high GC content or secondary structure may not dissolve rapidly. Heating at 55°C usually is sufficient to dissolve these oligos. Addition of DMSO gradually from a concentration of 1% to maximum concentration of 10% is recommended for oligos that do not dissolve even at 55°C.

Storage

Further dilution can be made in low TE buffer (10mM Tris, 0.1mM EDTA, pH 7 to 8.0). After reconstitution, store the stock solution at -80°C or -20°C. Fluorescently labeled oligos should be stored in light-free conditions.

Stability

Gene Link guarantees the stability of fluorescently modified oligos for 6 months if reconstituted and stored appropriately as recommended by Gene Link. The stability can be increased several fold by instituting proper handling conditions, avoiding exposure to light and multiple freeze thaws.

Antisense Oligos & SmartBase™ siRNA modified Oligos Stability, Reconstitution & Storage

Reconstitution

The dried oligo during transportation may have dislodged from the bottom of the tube and may reside in the cap and/or distributed on the sides of the tube.

Prior to opening the tube, briefly centrifuge the tubes to ensure that the dried oligonucleotide is at the bottom of the tube. Resuspend single stranded siRNA oligonucleotides at a convenient concentration, e.g. 100 µM, in RNase free sterile water and duplex siRNA at a concentration of 100 µM. These are general guidelines, and you may elect to reconstitute at a different concentration or buffer condition.

RNA & siRNA oligos especially single-stranded are susceptible to degradation by RNase introduced during handling. Wear gloves and use RNase-free pipette tips and RNase free conditions.

Antisense oligos, gapmers and SmartBase™ modified oligos that do not contain any native RNA bases are mostly stable as the unmodified oligos. These are supplied dried/lyophilized. These oligonucleotides should preferably be stored frozen upon receipt. TE (10mM Tris, 1mM EDTA, pH 8.0) is recommended for dissolving the oligonucleotides; EDTA inhibits the activity of the nucleases.

Further dilution can be made in low TE buffer (10mM Tris, 0.1mM EDTA, pH 7 to pH8.0). After reconstitution, store the stock solution at -80°C or -20°C.

Oligos can also be dissolved in nuclease free water if required. The pH of water should be between 7-8. Acidic water leads to depurination of oligos.

On occasion certain oligos with high GC content or secondary structure may not dissolve rapidly. Heating at 55°C usually is sufficient to dissolve these oligos. Addition of DMSO gradually from a concentration of 1% to maximum concentration of 10% is recommended for oligos that do not dissolve even at 55°C.

Storage

Further dilution can be made in low TE buffer (10mM Tris, 0.1mM EDTA, pH 7 to 8.0). After reconstitution, store the stock solution at -80°C or -20°C. Fluorescently labeled oligos should be stored in light-free conditions

Stability

Gene Link guarantees the stability of modified ASO and modified siRNA oligos for 6 months if reconstituted and stored appropriately as recommended by Gene Link. The stability can be increased several fold by instituting proper handling conditions, avoiding exposure to light and multiple freeze thaws.

Annealing of single-stranded siRNA

Reagents: Annealing buffer (5X)

Buffer 1:

50 mM Tris, pH 8.0
100 mM NaCl

Buffer 2:

100 mM Potassium Acetate
30 mM HEPES at pH 7.4
2 mM Magnesium Acetate

Note: Either Buffer can be used without much difference. All annealing solutions are given as 5X and can be stored frozen at -20ºC and freeze-thawed many times.

Annealing of single-stranded siRNA

1. Dissolve siRNA, as stated above, at a convenient concentration, e.g. 100 µM, in RNase free water and store at -20 ºC or preferably at -80ºC
2. Dilute each siRNA using sterile RNase free water to a final concentration of 50 µM.
3. Combine 30 µl of each siRNA solution and 15 µl of annealing buffer. Final volume is 75 µl, final concentration of siRNA duplex is 20 µM (30 µl X 50 µM = 75 µl X 20 µM).
4. Incubate the solution for 1 minute at 90 ºC and cool slowly down afterwards to room temperature (over a period of about 45 min). This can be conveniently performed using a thermal cycler.
5. Briefly spin the tube to bring down all droplets from the wall and lid of the tube.
6. Aliquot the annealed siRNA into RNase-free tubes and store at -80ºC. Do not freeze-thaw more than 5 times.

RNA and RNA modified Oligos Stability, Reconstitution & Storage

Reconstitution

The dried oligo during transportation may have dislodged from the bottom of the tube and may reside in the cap and/or distributed on the sides of the tube.

Prior to opening the tube, briefly centrifuge the tubes to ensure that the dried oligonucleotide is at the bottom of the tube. Resuspend single stranded RNA oligonucleotides at a convenient concentration, e.g. 100 µM, in RNase free sterile water and duplex siRNA at a concentration of 100 µM. These are general guidelines, and you may elect to reconstitute at a different concentration or buffer condition.

RNA oligos, especially single-stranded are susceptible to degradation by RNase introduced during handling. Wear gloves and use RNase-free pipette tips and RNase free conditions.

RNA oligos and RNA modified oligos are less stable as compared to DNA oligos and very susceptible to nuclease degradation. The presence of RNase on human skin/hands and bodily fluids makes the exposure to nuclease degradation more likely. Stringent laboratory nuclease free reagents and supplies should be used to reconstitute RNA oligos.

RNA and RNA Oligonucleotide Reconstitution and Storage Solution

RNA Reconstitution & Storage Solution (1 mM Sodium Citrate pH 6.4): Catalog No.: 40-5014-XX

Gene Link recommends Sodium citrate, 1 mM pH 6.4 as the RNA reconstitution solution. This is available from Gene Link.
Sodium citrate, 1 mM pH 6.4 is prepared in RNase free water and sealed in bottles after autoclaving. It is guaranteed RNase free and is the recommended solution for reconstitution and storage of RNA and RNA oligonucleotides. The low pH of 6.4 and low ionic strength of 1 mM Sodium citrate reduces base hydrolysis considerably and is an efficient chelating agent. Sodium citrate, 1 mM pH 6.4 as a reconstitution and storage solution is compatible with all RNA based applications.

RNA Reconstitution & Storage Solution (1 mM Sodium Citrate pH 6.4) is also available in convenient 10 tubes 1.6 mL aliquot packaging (Catalog No.: 40-5014-16) to meet the rigorous requirements of single use applications.

Content	Catalog No.	Description	Catalog No.	Unit Size
□	40-5014-16	RNA Reconstitution & Storage Solution (1 mM Sodium Citrate pH 6.4) 10 X 1.6 mL	40-5014-16	10 X 1.6 mL
□	40-5014-05	RNA Reconstitution & Storage Solution (1 mM Sodium Citrate pH 6.4); 50 mL	40-5014-05	50 mL

Alternatively, RNA oligos can also be reconstituted in nuclease free TE (10mM Tris, 1mM EDTA, pH 8.0; EDTA inhibits the activity of the nucleases.

Further dilution can be made in low TE buffer (10mM Tris, 0.1mM EDTA, pH 7 to pH8.0). After reconstitution, store the stock solution at -80°C or -20°C.

On occasion certain oligos with high GC content or secondary structure may not dissolve rapidly. Heating at 55°C usually is sufficient to dissolve these oligos. Addition of DMSO gradually from a concentration of 1% to maximum concentration of 10% is recommended for oligos that do not dissolve even at 55°C.

Storage
RNA oligos can be stored Sodium citrate, 1 mM pH 6.4 or low TE buffer (10mM Tris, 0.1mM EDTA, pH 7 to 8.0). After reconstitution, store the stock solution at -80°C or -20°C. For long term storage we recommend RNA should be stored as an ethanol precipitate at –80°C.

Stability
Generally, RNA oligos are stable for 6 months if reconstituted and stored appropriately as recommended by Gene Link. The stability can be increased several folds by instituting proper handling conditions, avoiding exposure to light and multiple freeze thaws.

For research use only. Not for use in diagnostic procedures for clinical purposes.

Document Warranty and Liability

Information in this document is subject to change without notice. This document and all information presented in this document are written as a guide. Gene Link, Inc. does not warrant this document to be free of errors and assumes no responsibility for any errors that may appear in this document.
Gene Link disclaims all warranties with respect to this document, expressed or implied, including but not limited to those of merchantability or fitness for a particular purpose. In no event shall Gene Link be liable, whether in contract, tort, warranty, or under any statute or on any other basis for special, incidental, indirect, punitive, multiple or consequential damages in connection with or arising from this document, including but not limited to the use thereof.

Website

As the receipt of information on the Internet is highly dependent upon factors, including without limitations to, the user's computer, browser, operation system, etc., information may be perceived incorrectly. Therefore, Gene Link does not warrant or guarantee that the information contained on its website www.genelink.com is error free.

Product Warranty and Liability

Warranty: Gene Link makes no warranty of any kind, specifically disclaims and excludes all other warranties of any kind or nature, directly or indirectly, express or implied, including, without limitation, as to the suitability, productivity, durability, fitness for a particular purpose or use, merchantability, condition, or any other matter with respect to Gene Link products. Gene Link products are for research purposes only including custom products. There is no warranty or claim of its performance for any specific research application. All Gene Link products are guaranteed to meet or exceed the specifications stated. Each Gene Link product is shipped with documentation stating specifications and other technical information. If the product fails to meet the stated specifications the sole remedy is prompt replacement by Gene Link or within 30 days of purchase a refund of the purchased price.

Liability: Under no circumstances shall Gene Link be liable for any damages directly or indirectly related to Gene Link's products and services. Whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Gene Link products to perform in accordance with the stated specifications.

Research Use Only. Not for use in diagnostic or clinical procedures.

Notice to Purchaser: The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact support@genelink.com

Gene Link, Inc.

www.genelink.com